AMENDMENT TO THE SPECIFICATION

Kindly amend the Abstract of the Specification as follows:

Abstract of the Disclosure

The present invention features methods and compositions for preventing, reducing, or treating hypoxia and pathological disorders involving abnormal angiogenesis (e.g., conditions involving decreases or increases in blood flow, respectively). ~~Thus, the present invention is useful for treating, reducing, or preventing ischemic conditions (characterized by a reduced blood flow or reduced angiogenesis, e.g., a myocardial infarct) and hypervascular conditions (characterized by excessive angiogenesis, e.g., cancer).~~ Where an increase in angiogenesis is desired, the mammal being treated for an ischemic condition is provided with Related Transcriptional Enhancer Factor-1 (RTEF-1; as a recombinant polypeptide or as an expression vector) sufficient to increase expression of VEGF, FGFR, or COX-2. This results in a concomitant increase in angiogenesis. Conversely, a mammal being treated for a hypervascular condition is administered a composition that reduces the levels of RTEF-1, thereby reducing the expression of VEGF, FGFR, or COX-2, which results in a decrease in angiogenesis. Also disclosed are screening methods that make use of RTEF-1 for the identification of novel therapeutics for the treatment, prevention, or reduction of pathological disorders involving hypoxia or abnormal angiogenesis, namely, ischemic or hypervascular conditions.

Kindly amend the Specification at page 4, lines 13-21, as follows:

According to this invention, when provided as a nucleic acid molecule, the RTEF-1 is encoded within an expression vector, such as plasmid or a viral vector (e.g., adenovirus, retrovirus, adeno-associated virus vector, herpes simplex virus, SV40 vector, polyoma virus vector, papilloma virus vector, picornavirus ~~picarnovirus~~ vector, or vaccinia virus vector). If desired, the RTEF-1-encoding nucleic acid molecule is under the control of a tissue-specific promoter. Consequently, RTEF-1 expression may be specific to any desired cell type including, for example, endothelial cells, cardiomyocytes, skin cells, hepatocytes, myocytes, adipocytes, and fibroblasts, as well as any cell type in any tissue in which the RTEF-1 is to be provided.

Kindly amend the Specification at page 14, line 21, through page 15, line 11, as follows:

As used herein, by "RTEF-1" is meant any polypeptide that exhibits an activity common to its related, naturally occurring RTEF-1 polypeptide (Accession Numbers AAC50763 (SEQ ID NO: 7), Q62296, P48984, or Q62296), preferably in its activated form. Accordingly, the RTEF-1 of the invention is substantially identical to the naturally occurring RTEF-1 (at least 60%, 70%, 80%, 85%, 90%, 95%, or more than 100% identical to the human, mouse, or chick RTEF-1), and when administered, the RTEF-1 has angiogenic activity. Desirably, the RTEF-1 having RTEF-1 biological activity binds the VEGF, FGFR, or COX-2 promoter and induces transcription of the VEGF, FGFR, or COX-2 gene. Preferably, the RTEF-1 increases VEGF, FGFR, or COX-2 transcription by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more than

100% above control levels as measured by any standard method known in the art or described herein. Alternatively, the RTEF-1 of the invention increases angiogenesis by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more than 100% above untreated control levels as measured by any standard method in the art or described herein. RTEF-1 is described, for example, in Stewart et al., (1996) *Genomics* (36): 68-76, U.S.P.N. 5,776,776, and Ueyama et al., J. Biol. Chem. 275:17476-17480, 2000, all of which are hereby incorporated by reference. Regions of RTEF-1 that have structural significance for biological function include, e.g., the DNA binding domain at the amino-terminal end of RTEF-1, as is discussed in Ueyama et al., serine residues at the carboxy-terminus of RTEF-1 (e.g., Ser 254, Ser-290, Ser-322, and Ser 358 relative to the human wild-type sequence) the mutation of which has been shown to result in loss of RTEF-1 phosphorylation via interaction with PKC and MAPK and concomitant loss of signaling ability, and the STY domain of RTEF-1 (amino acids 299-358, relative to the human sequence)).

Kindly amend the Specification at page 15, line 19, through page 16, line 8, as follows:

By "substantially identical," when referring to a protein or polypeptide, is meant a protein or polypeptide exhibiting at least 75%, but preferably 85%, more preferably 90%, most preferably 95%, or even 99% identity to a reference amino acid sequence. For proteins or polypeptides, the length of comparison sequences will generally be at least 20 amino acids, preferably at least 30 amino acids, more preferably at least 40 amino acids, and most preferably 50 amino acids or the full length protein or polypeptide. Nucleic acids that encode such "substantially identical" proteins or polypeptides constitute an example of "substantially

identical" nucleic acids; it is recognized that the nucleic acids include any sequence, due to the degeneracy of the genetic code, that encodes those proteins or polypeptides. In addition, a "substantially identical" nucleic acid sequence also includes a polynucleotide that hybridizes to a reference nucleic acid molecule under high stringency conditions. Methods to determine identity are available in publicly available computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GCG program package (Devereux et al., *Nucleic Acids Research* 12: 387, 1984), BLASTP, BLASTN, and FASTA (Altschul et al., *J. Mol. Biol.* 215:403 (1990). The well-known Smith Waterman algorithm may also be used to determine identity. The BLAST program is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, et al., NCBI NLM NIH, Bethesda, MD 20894~~; BLAST 2.0 at http://www.ncbi.nlm.nih.gov/blast/~~). These software programs match similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications.

Kindly amend the Specification at page 19, lines 7-9, as follows:

FIGURE 3B is a schematic diagram showing the consensus sequence of transcription factor binding sites (Sp1, Egr1, and AP2 elements) in the VEGF promoter sequence of –100/–50 (SEQ ID NO: 4).

Kindly amend the Specification at page 21, line 21, through page 22, line 2, as follows:

FIGURE 9 is a schematic diagram showing various truncated FGFR1 promoters operably linked to a luciferase reporter gene and the promoter activity associated with each mutant. Transient transfection assays were performed in BAEC cells using a set of truncated FGFR1 promoter Luc constructs and control vector pcDNA3.1/GS or an equal amount of RTEF-1 cDNA. The activity of FGFR1 promoter constructs, 0.99kb, 0.44kb, 0.28kb, and 0.15kb (-48 ~ +94 related to the 5' transcriptional starting site) was found to increase over three fold in the presence of RTEF-1, but almost no stimulated activity was observed on the 0.12 kb construct (-20 ~ +94). RTEF-1 binding of the FGFR1 promoter was associated with the -48 to -21 region of the FGFR1 promoter (SEQ ID NO: 5).

FIGURE 10 is a schematic diagram showing deletion and mutation analysis of the 0.15 kb FGFR1 promoter (-48/+94). The sequence between -48 and -20 of the FGFR1 promoter contains a stimulating protein (SP)-1 like element and is responsible for the RTEF-1 protein binding (SEQ ID NO: 6). Interestingly, there is no M-CAT related binding element found within this region.

Kindly amend the Specification at page 22, line 14, as follows:

FIGURE 13 provides the sequence for human RTEF-1 (SEQ ID NO: 7).

Kindly amend the Specification at page 46, lines 4-25, as follows:

To identify the specific regulatory element on the VEGF promoter that is bound by RTEF-1, we performed *in vitro* transcription/translation labeling with ^{35}S-methionine to express RTEF-1 product from a RTEF-1 cloned construct. As shown in FIGURE 6A, the translated product of RTEF-1 cDNA generated the expected protein size of 54 KD. Next, we created a series of double-stranded oligonucleotide probes corresponding to the individual VEGF promoter Sp1 sequences for use in electrophoretic mobility shift assays (EMSA). The VEGF Sp1-I, -II, and -III oligonucleotide sequences were used to generate radiolabeled probes for use in competition assays. In addition, two mutated double-stranded oligonucleotides, in which either the Sp1 consensus sequence was substituted with **ttttttttttt** (Mut 1, -97/-87; SEQ ID NO: 1) or the **CC** of the core Sp1 sequence was substituted with **tt** (Mut 2, -92/-91), were used to determine the specificity of RTEF-Sp1 binding. As shown in FIGURE 6B, RTEF-1 was found to bind to the Sp1-I motif on the VEGF promoter by EMSA (specified by anti-RTEF-1 antiserum, Band SS); there was no band at the same position in the Sp1-II, Sp1-III, Ap2, and Egr1-I labeled complex. This RTEF/Sp1-I complex was specific because it was repressed by the addition of excessive unlabeled Sp1-I oligonucleotide. Moreover, the DNA–protein complex (band C) was not eliminated by the mutant Sp1 consensus oligonucleotide (Mut 1), while the mutated core Sp1 oligonucleotide (Mut 2) was able to compete with hot Sp1-I oligonucleotides for binding to the RTEF-1 product, thereby out-competing some of the DNA-protein complex labeled band. No DNA–protein complex was identified when mutated Sp1 consensus oligonucleotide (Mut 1) was

used as a probe.

Kindly amend the Specification at page 54, lines 24-30, as follows:

Total RNA from BAEC cells was extracted using TRI Reagent (Sigma) according to the manufacturer's protocol, and was electrophoresed on a 1.3% agarose/6% formaldehyde gel. Hybridization was analyzed under stringent conditions with human RTEF-1 cDNAs radiolabelled with ^{32}P-dCTP, using the Klenow fragment of DNA polymerase I and random oligonucleotides as primers (Promega, Madison, WI). The blots were washed and autoradiograms were developed after exposure to X-ray film at –70°C, using a CRONEX™ ~~Cronex~~ intensifying screen (DuPont, NY).

Kindly amend the Specification at page 57, lines 2-16, as follows:

Chromatin immunoprecipitation assays were performed according to the protocol from Dr. Farnham's laboratory (Weinmann et al., Mol. Cell Biol. 21:6820-6832, 2001; Eberhardy et al., J. Biol. Chem. 275:33798-33805, 2000; Eberhardy et al., J. Biol. Chem. 276:48562-48571, 2001) with the following modifications. Briefly, immunoprecipitation (IP) of transfected and formaldehyde-cross-linked BAEC cells was performed overnight at 4°C in the presence of RTEF-1 antiserum. Fifty percent of the supernatant from the RTEF-1 antiserum-free IP was saved as "total input" chromatin and processed with the eluted IPs beginning with the reverse of

formaldehyde-cross-linking. After final ethanol precipitation, the TE resuspended IP products were used as templates for PCR using two primers (5'-GCTGAGGCTCGCCTGTCCCCGCCCC-3' (SEQ ID NO: 2) and 5'-CAAATTCCAGCACCGAGCGCCCTGG-3' (SEQ ID NO: 3)). These two primers were designed according to the sequences 5' and 3', respectively, to the putative Sp1 binding domain on the proximal portion of the VEGF promoter. 1× dilution buffer was used as a negative control (mock) for PCR.